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ABSTRACT

Precise and accurate cholesterol measurements are required to identify and treat individuals with high blood cholesterol levels. However, the current state of reliability of blood cholesterol measurements suggests that considerable inaccuracy in cholesterol testing exists. This report describes the Laboratory Standardization Panel findings on the precision and accuracy of plasma or serum total cholesterol measurements in United States clinical laboratories and provides a series of broad recommendations designed to improve performance. The report is divided into 5 sections concerning: (1) why precise and accurate cholesterol measurements are needed; (2) current state of reliability of blood cholesterol measurement: the clinical laboratory, the physicians office and alternate site testing equipment; (3) reliable cholesterol measurements: what is possible; (4) factors contributing to acceptable analytical performance; and (5) resources currently available. A glossary and 28 references are included. (JD)

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Current Status of

BLOOD CHOLESTEROL MEASUREMENT

in

Clinical Laboratories in the United States

A REPORT FROM THE LABORATORY STANDARDIZATION PANEL OF THE NATIONAL CHOLESTEROL EDUCATION PROGRAM

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NATIONAL CHOLESTEROL EDUCATION PROGRAM

National Cholesterol Education Program
Coordinated by the
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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A. *Overview and Recommendations*

A successful national effort to identify and treat every American adult at high risk for coronary heart disease (CHD) because their blood has a high cholesterol concentration is expected to contribute to lower CHD morbidity and mortality rates. All adults need to know their blood cholesterol level, to be aware of the implications of elevated cholesterol, and to seek the help of a physician should treatment be necessary.

Precise and accurate cholesterol measurements are required to identify and treat individuals with high blood cholesterol levels. However, the current state of reliability of blood cholesterol measurements made in the United States suggests that considerable inaccuracy in cholesterol testing exists. As part of the unified effort to identify and treat the one in four American adults at substantially higher risk for CHD, the National Cholesterol Education Program and its Laboratory Standardization Panel on Blood Cholesterol Measurement are developing recommendations to improve laboratory performance. This report provides an overview of the Laboratory Standardization Panel's assessment of the current situation and a brief outline of the Panel's preliminary recommendations to improve laboratory performance.

Adoption of the following broad recommendations should lead to considerable improvements in the quality of cholesterol measurement:

- Accurate and precise cholesterol measurements are needed for the uniform interpretation of cholesterol values to assess a person's risk for CHD and to monitor treatment.
- All adults should know their cholesterol level, be aware of the implications of elevated cholesterol in regard to the increased risk of coronary heart disease, and seek the help of a physician should further evaluation and treatment be necessary.
- All clinical laboratories in the United States should adopt uniform cholesterol cutpoints for identifying adults at high risk for CHD. The Laboratory Standardization Panel recommends adoption of the cutpoints that were issued by the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults in October 1987. This requires national standarization of cholesterol measurements. In order to use the new recommended cutpoints properly, a laboratory must minimize method-specific biases and also achieve adequate precision of cholesterol measurement. Specific attention to method-instrument and calibration procedures is necessary to minimize method-specific biases.

- Bias (deviation from the true value) of cholesterol measurement methods currently in use should not exceed ±5% from the true value and should be no greater than ±3% from true value within 5 years. Data from proficiency testing surveys suggest that many clinical laboratories need to improve the overall reliability of cholesterol measurements. About half of the clinical laboratories that participated in a survey exceeded 5% from true value (the minimal acceptable accuracy performance recommended by the panel members). *Precision* appears to be less of a problem.
- The newly available portable chemistry analyzers for cholesterol measurement should have further evaluation before they are adopted for routine use with patients. In addition, proper training of technical personnel in the use and maintenance of these new analyzers and in the proper use of quality assurance procedures is essential.
- Cholesterol measurements made by all clinical laboratories in the United States should and can be standardized so that the cholesterol values are *traceable* to the Centers for Disease Control (CDC) reference method or to the National Bureau of Standards (NBS) definitive method. Laboratories can accomplish this goal and also improve the accuracy and precision of their cholesterol measurements by using certified reference materials currently available from NBS, CDC, or the College of American Pathologists (CAP) to evaluate their cholesterol measurement methods and/or instruments.
- Modifications may be necessary in some reagent and instrument systems to obtain adequate specificity for cholesterol measurement and to minimize the effect of interfering substances.

Acceptable performance is attainable with the adoption of these recommendations: most of the resources necessary to achieve reliable performance are already available. Certified reference materials for assessing accuracy are available from NBS, CDC, and CAP.

A subsequent report from the Laboratory Standardization Panel will recommend the technical and organizational elements needed to assure the overall reliability of cholesterol measurement.

B. *Introduction*

Awareness is growing that reduction of elevated blood cholesterol levels is important for the prevention of coronary heart disease (CHD). While this heightened awareness can generally be attributed to a gradual increase in understanding of the role of cholesterol in atherogenesis gained from numerous studies over the past 20 to 30 years, several recent events are particularly responsible. These include the report of the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT), the recommendations of the National Institutes of Health (NIH) Consensus Development Conference on Lowering Blood Cholesterol to Prevent Heart Disease, the award of the 1985 Nobel Prize in Medicine to Dr. Michael Brown and Dr. Joseph Goldstein for their work on the low density lipoprotein (LDL) receptor, and the recent initiation of the National Cholesterol Education Program by the National Heart, Lung, and Blood Institute (NHLBI).

The LRC-CPPT, a multicenter, randomized, double-blind study, was initiated in 1973 to determine the efficacy of cholesterol lowering in reducing the risk of CHD in middle-aged men with primary hypercholesterolemia. The treatment group received the bile acid sequestrant, cholestyramine, and achieved an 8.5% greater reduction in cholesterol levels than did the placebo group (1,2). The cholestyramine-treated group experienced a 19% reduction in definite CHD death and/or definite nonfatal infarction. The incidence rates for new positive exercise tests, angina, and coronary bypass surgery in the cholestyramine group were also significantly reduced by 25%, 20%, and 21%, respectively. Moreover, the incidence of CHD in men who sustained a decrease of 25% in total cholesterol was almost 50% lower than that of men whose total cholesterol remained at pre-treatment levels.

The NIH Consensus Development Conference on Lowering Blood Cholesterol was charged in 1984 with reviewing the evidence relating cholesterol levels to CHD. The Consensus Conference Panel unanimously concluded that elevated blood cholesterol is a major cause of coronary artery disease and that lowering elevated blood cholesterol levels (specifically blood levels of LDL) will reduce the risk of heart attacks attributable to CHD, as established by the findings of the LRC-CPPT and related studies (3). After careful review of genetic, experimental, epidemiologic, and clinical trial evidence, the Consensus Conference Panel recommended classifying and treating substantially higher-risk adults, that is, those with blood cholesterol above the 75th and 90th percentiles (3).

In November 1985 the National Cholesterol Education Program was initiated by NHLBI with the goal of decreasing the prevalence of elevated blood cholesterol in the United States, in order to help reduce CHD morbidity and mortality rates. A major objective of the program is to identify and treat the one in four American adults at significantly increased risk for the development of CHD because of high cholesterol levels. As part of the overall cholesterol education program, two panels of experts were established in the earliest phase of the effort: the Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel) and the Laboratory Standardization Panel on Blood Cholesterol Measurement.

The Adult Treatment Panel's charge was to develop practical and detailed guidelines for clinicians to use in measuring, assessing, and treating high blood cholesterol in adult patients. The Panel developed recommendations for total cholesterol and LDL-cholesterol cutpoints that slightly modify the Consensus Development Conference cutpoints and that require accurate measurements of total cholesterol, high density lipoprotein (HDL)-cholesterol, and triglycerides (Table 1) (4).

Table 1. Recommendations of the Adult Treatment Panel of the National Cholesterol Education Program for Classification of Patients (4)

Classification Based on Total Cholesterol	Classification Based on LDL-Cholesterol
<200 mg/dL (<5.17 mmol/L) Desirable Blood Cholesterol	<130 mg/dL (<3.36 mmol/L) Desirable LDL-Cholesterol
200-239 mg/dL (5.17-6.18 mmol/L) Borderline-High Blood Cholesterol	130-159 mg/dL (3.36-4.11 mmol/L) Borderline-High-Risk LDL-Cholesterol
≥240 mg/dL (≥6.21 mmol/L) High Blood Cholesterol	≥160 mg/dL (≥4.13 mmol/L) High-Risk LDL-Cholesterol

* To convert mg/dL cholesterol to mmol/L: divide cholesterol by 38.7 or multiply by 0.02586.

Given the important role that determinations of cholesterol levels play in the prevention and management of coronary heart disease, the National Cholesterol Education Program regards it as important that clinical laboratories in the United States provide accurate and precise cholesterol measurements, and that the Laboratory Standardization Panel review and evaluate the current state of reliability of cholesterol testing, promote the uniform interpretation of laboratory results, and recommend means to improve precision and accuracy of cholesterol analyses. The membership of the Laboratory Standardization Panel includes laboratory experts and representatives of major clinical laboratory professional groups, Federal governmental agencies, and industry.

The need for reliable laboratory measurements of plasma or serum lipids is not new and will grow as the demand for cholesterol testing markedly increases (5-13). The primary purpose of this report is to review the current state of cholesterol measurement in U.S. clinical laboratories and to evaluate the potential for improving performance in the measurement. A more detailed and comprehensive report will follow containing recommendations designed to improve the measurement of cholesterol. Because the detection and treatment guidelines from the Adult Treatment Panel will go beyond the measurement of total cholesterol, to the measurement of LDL-cholesterol as well, it is also essential that accurate and precise measurements of triglycerides and HDL-cholesterol be made so that LDL-cholesterol values can be reliably estimated; the LDL-cholesterol value will be the key determinant upon which a clinical decision will be based to intervene with cholesterol-lowering therapy. These analytical constituents will be examined in subsequent separate reports. This report emphasizes the need for reliable measurements in the total cholesterol concentration range.

C. *Why Precise And Accurate Cholesterol Measurements Are Needed*

Cholesterol measurements are made in the clinical laboratory to detect individuals with hypercholesterolemia, to confirm or diagnose the pattern of hyperlipidemia, and to monitor the changes in cholesterol levels as the result of treatment. Two aspects of analytical performance in laboratory measurements are essential: *precision* and *accuracy*. Analytical methods must be precise; that is, the methods must be reproducible so that variability in repeated measurements of the same sample is within acceptable limits. Measurements must be accurate; that is, the measured value must agree with the "true value," within acceptable limits.

Precision or reproducibility is an important and integral component of reliable cholesterol measurements. Consistent accuracy is not possible if the measurements are imprecise. Thus, initial efforts should be focused on achieving a cholesterol assay system that is adequately precise. As illustrated in Figure 1, an imprecise analytical system gives results scattered over a wide concentration range when the same sample is analyzed on several occasions. Replicate measurements of a specimen with a true cholesterol value of 240 mg/dL (6.21 mmol/L) could be expected to be distributed such that 95% (± 2 S.D.) of the observations fall within the range of 192 to 288 mg/dL (4.96 to 7.44 mmol/L), when a method with a 10% relative standard deviation or coefficient of variation (C.V.) is used. Such an imprecise method makes it impossible to measure cholesterol reliably or to follow the changes in the patient's cholesterol level to monitor the effectiveness of treatment. For example, current cholesterol-lowering diets may be expected to lower blood cholesterol on the average by 10-15%. A patient's initial cholesterol level of 250 mg/dL (6.47 mmol/L) might decrease to 225 mg/dL (5.82 mmol/L) as a result of dietary treatment. This 10% change may not be detected by a method with poor precision. Reliable cholesterol measurements are absolutely essential for an effective CHD reduction program. *The Laboratory Standardization Panel recommends that, as a national goal, clinical laboratories should initially achieve an overall precision consistent with a C.V. of 5% or less; ultimately, laboratories should achieve a C.V. of 3% or less.*

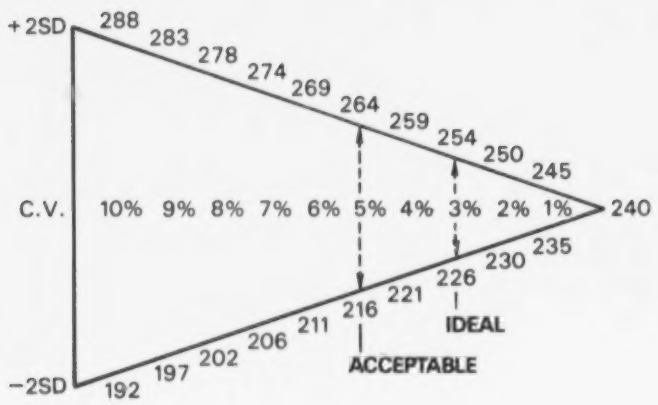

Figure 1. The effect of differing degrees of analytical imprecision of cholesterol measurement at a medical decision point of 240 mg/dL (6.21 mmol/L) (as true value). Acceptable precision goal = 5% or less from true value; ideal goal = 3% or less from true value.

Accuracy, agreement with the true value, is also essential to a reliable analytical system. Quantitative measurements are relative in nature; that is, the measurements are based upon comparison with a reference material with a known concentration of analyte that has been previously established by a reference and/or definitive method. A variety of cholesterol measurement methods are in use, each having unique performance characteristics. The use of different *methods* (each based on different *analytical principles*, or which use different *reagents*, *calibrators*, and *instruments*) for the same method can lead to inaccuracy or biases. *Laboratories must give special attention to methods and calibration procedures to minimize the method-instrument-specific biases.*

Inaccurate measurement may lead to clinical misdiagnosis because of the reporting of false positive or false negative values. For example, a laboratory that measures cholesterol with a method having a positive bias of 10% at a medical decision point of 200 mg/dL (5.17 mmol/L) would report the falsely high value of 220 mg/dL (5.69 mmol/L). With the same percentage bias at the 240 mg/dL (6.21 mmol/L) decision point for high risk (Table 1), the laboratory would report a falsely high cholesterol result of 264 mg/dL (6.83 mmol/L). Thus, the higher the *true* concentration of the blood cholesterol, the greater the absolute magnitude of the error; this magnifies the unreliability of the values. *The Laboratory Standardization Panel recommends that biases in methods presently in use should not exceed ±5% from the true value and that ultimately, a national goal of ≤3% bias should be achieved.* It is not uncommon for clinical laboratories in the United States to have cholesterol-method biases exceeding ±5 to ±10%, or even ±15% or more. In previous years such biases were tolerated because each laboratory

established and used its own "normal" or reference range. A recent nationwide survey of 152 academically based clinical laboratories showed that reference ranges vary widely among laboratories (15). The reference ranges used by different laboratories are not only diverse, but in many instances high, compared with the current guidelines (Table 1). Some institutions still report the upper limits of "normal" as 330 mg/dL (8.53 mmol/L), or even 350 mg/dL (9.05mmol/L). *It is recommended that all laboratories adopt a uniform method of reporting cholesterol values relating to risk for coronary heart disease. The Laboratory Standardization Panel recommends adoption of the cutpoints that were issued by the National Cholesterol Education Program Adult Treatment Panel in October 1987 (Table 1). In order to utilize the new cutpoints properly, it is recommended that a laboratory minimize the method-specific biases and also achieve adequate precision.*

D. *Current State of Reliability of Blood Cholesterol Measurement*

1. The Clinical Laboratory

The difficulty in obtaining reliable clinical laboratory measurements of blood cholesterol has been documented over the last 4 decades through various proficiency testing surveys (16). The ability of such external quality surveillance programs to assess historical trends in the state of the art is well established (8,17-19). The national surveys of the College of American Pathologists (CAP), which began in the 1940s, have evolved into the largest proficiency testing program in the world. The program's primary purpose is to promote laboratory improvement through voluntary educational and peer comparison programs. Various CAP programs survey nearly 10,000 laboratories or about 70% of all U.S. clinical laboratories. The survey data can be used to assess accuracy and both *intra(within)laboratory* and *inter(among)laboratory* variability for the major cholesterol methods.

The CAP Quality Assurance Service (QAS) program provides statistical analyses and charting of quantitative data generated from quality control (QC) pools analyzed routinely on a daily basis along with patient samples. Currently about 2,000 laboratories participate in the QAS programs for cholesterol in which control materials are routinely run in internal quality control procedures with patient samples. Because participating laboratories analyze the same QC pools repeatedly over long periods of time, the QAS data can be used to derive estimates of short-term and long-term *precision* within laboratories in addition to variation among laboratories (17).

Estimates of average long-term (day-to-day) precision within a laboratory are shown in Table 2. In 1985, the *intralaboratory* variability was about 3.5% (C.V.), slightly improved over 1975 and 1980. With the majority of the participating laboratories achieving C.V.'s of 3.5% or less, intralaboratory precision does not appear to be a major problem for most laboratories.

Table 2. National Trends in Intralaboratory Precision For Cholesterol Measurements*

	1975	1980	1985
Cholesterol	4.1%	3.8%	3.5%

Source: CAP-QAS data (from CAP Computer Center, Traverse City, Michigan)

* Mean precision (C.V.) after exclusion of outliers (submitted values exceeding $\bar{x}$ ±3 S.D.).

The *interlaboratory* component of variability is an indication of method accuracy among the participating laboratories. Historical trends in this parameter are illustrated in Table 3. The interlaboratory variability has improved substantially since 1947, and in 1986 an overall C.V. of ±6.2% was achieved. This substantial improvement in *interlaboratory comparability* is probably attributable, in part, to the increasing use of more specific enzymatic reagents and better automated chemistry analyzers. The interlaboratory component is considerably larger than the intralaboratory component, suggesting that method- and laboratory-specific biases are major contributors to the overall variability in cholesterol analyses. *This source of variability can be reduced by improving calibrating procedures.*

Table 3. National Trends in Interlaboratory Comparability*

	1949	1969	1980	1983	1986
Cholesterol	23.7%	18.5%	11.1%	6.4%	6.2%

Source: CAP-QAS data (from CAP Computer Center, Traverse City, Michigan)

* Mean precision (C.V.) after exclusion of outliers (submitted values exceeding $\bar{\bar{x}}$ ±3 S.D.).

Another major CAP survey of laboratory performance in cholesterol analysis is the Chemistry Proficiency Testing Survey. Approximately 5,000 laboratories participate each quarter in this Comprehensive Chemistry Survey with measurements of two pools of serum with unassigned cholesterol values. The results of a recent CAP survey (1985 Comprehensive Chemistry Survey, Set C-17), shown in a quarterly summary report in Table 4, may be used as a representative example to assess the present state of cholesterol measurement by clinical laboratories in the United States. Based on instruments and methods, 28 peer groups are listed; a peer group requires at least 20 laboratory participants. Twenty-four additional method-instrument combinations, with insufficient participants for a peer group, are not included in Table 4. It is apparent that many different analytical procedures are currently used to measure total cholesterol. The results on specimen C-17, submitted by 5,004 participating laboratories, ranged from 101 to 524 mg/dL (2.61 to 13.54 mmol/L). After the removal of 107 outliers (≥ ±3 S.D. from the overall laboratory mean value), the remaining cholesterol values (which include *nonenzymatic* and *enzymatic* methods) ranged from 182 mg/dL to 379 mg/dL (4.70 to 9.79 mmol/L). When only the participants using enzymatic

methods (97%) are reviewed (Figure 2), the range of submitted values is somewhat better (205 mg/dL to 309 mg/dL; 5.30 mmol/L to 7.99 mmol/L). Similar data are observed in the 1986 and current 1987 CAP surveys; thus, the 1985 data reflect the current status of cholesterol measurements in U.S. clinical laboratories.

To assess *accuracy*, two comparative values are currently provided in the CAP Comprehensive Chemistry quarterly report. One is the "all method-principle result," a consensus mean value that is the average of all results reported by the CAP participants. The second is the CDC Confirmatory Value, determined by the Centers for Disease Control (CDC)[1], using a modified Abell-Kendall method, which is accepted as the *reference method* for cholesterol (19-21). The two comparative values differ usually by not more than 1.5%. A recent joint National Bureau of Standards (NBS)-CAP report (18) confirms Gilbert's initial observation (19) that the "all method-principle result" consensus mean values are reasonably accurate, agreeing within 1% of the values obtained on survey pools by an NBS method, accepted as providing definitive values for cholesterol. In 1978, a CDC and NBS comparison study of the reference and definitive methods on five serum pools gave results that agreed within 0.5-0.7% on all pools. These studies suggest that both the CAP "all method-principle result" mean values and the CDC Confirmatory Values can be used as reliable means of assessing laboratory performance in terms of accuracy.

Figure 2 depicts the distribution of cholesterol results on specimen C-17 by participants who use enzymatic methods after the removal of outlier values. More than 47% of the results were equal to or greater than ±5% from the CDC Confirmatory Value; of these 47%, about 16% and 8% were equal to or greater than ±10% and ±15%, respectively. With only a single measurement by each surveyed laboratory, the observed variability must be attributed to both inaccuracy, or bias, and imprecision.

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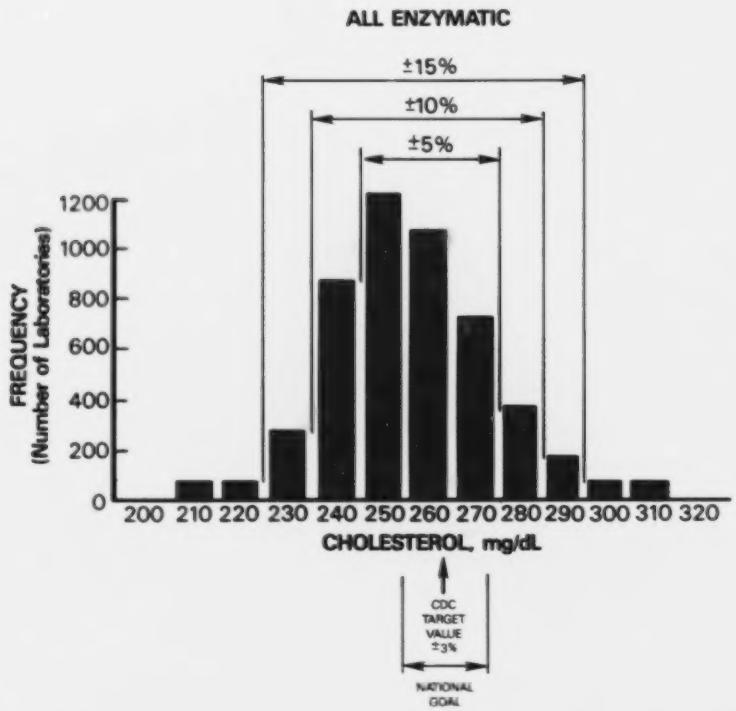

Figure 2. Distribution of cholesterol values of Set C-17 by CAP participants utilizing enzymatic technology. CDC target value for Set C-17 is 262.6 mg/dL (6.79 mmol/L).

As indicated previously inaccuracy appears to be the major problem. It should be noted that certain peer groups exhibited more variability (large standard deviations from the mean), suggesting less uniformity with some instrument-method combinations than with others (Table 4). Comparison of the mean for each peer group with the CDC Confirmatory Value indicates that some peer groups showed less bias than others. However, even in some of the "better" peer groups, a large standard deviation (shown as ±1 S.D. in Table 4) indicates that some of the individual laboratories did not report accurate results. The peer groups with significant bias (> ±5%) from the CDC Confirmatory Value have serious accuracy problems.

2. Physician Office and Alternate Site Testing Equipment

A new generation of simple-to-operate chemistry analyzers is being introduced by many manufacturers. This new technology makes it possible for many clinical chemistry tests traditionally performed in a laboratory, including cholesterol measurements, to be done elsewhere–for instance, in outpatient clinics, physicians' offices, and at cholesterol screening sites such as shopping malls,

Table 4. CAP 1985 Comprehensive Chemistry Survey (Cholesterol Data)

CHOLESTEROL (mg/dL) Method/System	Specimen C-17 No. Labs	Mean	±SD	C.V.[2] (%)	Range[3]	OL[2]
All method-principle						
All instruments	5004	257.2	17.3	6.7	182-379	107
Liebermann-Burchard						
With Extraction	23	261.7	17.2	6.6	331-300	–
Without Extraction	92	258.1	18.9	7.3	190-308	–
Liebermann-Burchard (Without Extraction)						
Technicon SMAC	20	255.7	14.4	5.6		
Enzymatic						
Abbott 50	11	255.5	10.0	3.9	244-274	0
Abbott ABA 100	52	254.9	13.2	5.2	224-288	3
Abbott ABA 200	39	240.9	21.1	8.8	182-279	0
Abbott VP	254	248.1	11.8	4.8	210-281	10
American Monitor Parallel	62	248.1	10.6	4.3	221-379	1
American Monitor KDA	238	260.6	12.5	4.8	231-299	5
Ames Seralyzer	49	281.2	38.5	13.7	187-372	0
Aminco Rotochem	39	252.7	14.2	5.6	215-294	1
Baker	221	247.0	16.7	6.8	206-361	8
Beckman Astra 4&8[4]	165	334.1	17.4	5.2	267-397	8
Boehringer Mann. Diag. 8700M	76	251.3	10.8	4.3	223-289	3
Chemetrics II	107	258.0	19.4	7.5	207-314	4
Coulter DACOS	109	268.4	9.8	3.7	239-291	2
DuPont ACA	1005	246.6	7.7	5.1	222-270	27
Electronucleonics Flexigem	32	243.4	13.5	5.6	212-278	3
ElectroNucleonics Gemeni	271	248.5	16.6	6.7	197-296	7
Gilford Impact 400, etc.	196	280.0	21.3	7.6	224-346	3
Gilford Sys 102	64	277.4	23.0	8.3	224-337	1
Gilford Sys 103, 202, 5	74	271.1	16.1	5.9	234-317	3
Hitachi 705 (BMD)	264	258.9	5.8	2.2	241-274	8
I.L. Multistat III	157	249.7	16.9	6.8	209-300	3
Kodak Ektachem	224	269.8	10.0	3.7	241-300	4
Olympus Demand	120	254.7	9.8	3.8	224-279	3
Roche Cobas	140	262.6	18.1	6.9	212-311	4
Technicon RA 1000	175	262.7	10.4	4.0	237-293	3
Technicon SMA 12/60	206	263.1	10.4	4.0	232-287	10
Technicon SMAC	231	271.6	7.1	2.6	250-294	4
All multiconstituent analyzers	4570	256.5	16.8	6.5		
CDC Confirmatory Result Value	1	262.6	1.5	0.49		

1 From The College of American Pathologists Survey Data presented in their CAP 1985 Comprehensive Chemistry Study (Cholesterol Data), SUMMARY REPORT.

2 C.V. = coefficient of variation, OL = outliers.

3 Range = after removal of outliers.

4 For two consecutive quarters this peer group reported falsely elevated values that may have been due to matrix effect with selective lots of CAP survey materials. The manufacturer has since reformulated its reagents to minimize or eliminate this effect. All subsequent survey results indicate that this problem has been eliminated with this peer group.

schools, and churches. These analyzers have the advantages of being economical, compact, lightweight, easy-to-operate, convenient, and requiring small sample volume. Some systems utilize fingerstick specimens coupled with chemistry tests based on dry reagent technology (as either multi- or single-layered films) or other innovative technology.

On the basis of preliminary assessments, some of these analyzers have the potential for providing accurate and precise cholesterol results similar to those from the more sophisticated analyzers used in the large clinical laboratories. However, there have also been reports of problems associated with some of these new analyzers. While potentially useful, these first-generation analyzers need more thorough evaluation and programs must be developed to assure acceptable performance. Users need to understand the performance characteristics and limitations of the new analyzers before adopting them for routine use with patients. Various studies, some sponsored by manufacturers, are seeking to meet these needs. Of particular interest is an NHLBI study, "Model Systems for Blood Cholesterol Screening," which is currently evaluating the reliability of cholesterol measurements done in the field with such analyzers.

In addition to the requirement for reliable testing equipment and reagent systems, the proper technical training of personnel in the use and maintenance of these new analyzers and in the proper use of quality assurance procedures is essential. Since these instruments are designed to be used by individuals who are not primarily trained in laboratory analysis, operators must be especially well trained in such areas as the proper drawing of blood, handling and storage of samples, effects of biological and environmental factors, proper instrument maintenance, and adequate quality control procedures. These considerations are necessary to obtain reliable measurements in both the clinical and non-clinical laboratory settings, including physicians' offices. A subsequent report will provide greater detail about these issues to help ensure more reliable cholesterol measurements.

E. *Reliable Cholesterol Measurements: What Is Possible*

It is possible to measure cholesterol accurately and precisely. An example of what can be achieved is given by the experience of the laboratories of the Lipid Research Clinics (LRC) program.

The LRC laboratories minimized analytical bias and variability of cholesterol results by requiring that all laboratories use the same instrumentation, the same (*nonenzymatic*) cholesterol method, and the same primary standard solutions, secondary calibration standards (sera), and quality control sera with established target values traceable to the CDC reference method (22). In addition, the common rigorous protocol used by all laboratories included a well-defined, thorough internal quality control program and an external quality control surveillance program. Because of this *standardization* effort, an overall bias of −1.3% was achieved when the 12 LRC laboratories were compared with the CDC reference values (23,24). Precision within the laboratories was also excellent: an overall day-to-day reproducibility of ≤3% C.V. was maintained over many years.

Similar results were achieved in the Air Force HEART Study (25). Using an automated laboratory instrumentation and a state-of-the-art *enzymatic* method in a single core laboratory, the study obtained an overall mean bias of <1.2% (when compared to CDC values) and an average day-to-day precision of <0.7% C.V.

F. Factors Contributing to Acceptable Analytical Performance

Factors contributing to acceptable *precision* are basic components of accepted laboratory practice and quality assurance. Attention to these factors will assure acceptable precision in cholesterol measurement in the clinical laboratory. Some of these factors are:

- overall commitment to quality performance;
- analytical methods based on sound and well-established analytical principles with effective and stable reagents;
- reliable instrumentation with good performance characteristics and thorough maintenance programs;
- rigid instrument maintenance schedules with well-established protocols that require complete documentation;
- effective quality control programs;
- competent, well-trained, and motivated staff to perform the tests;
- uniform specimen collection, handling, and storage; and
- mechanisms for identifying and correcting problems.

Obtaining *accurate* cholesterol measurements requires additional efforts. *The use of reference materials with accurate target values for calibration and monitoring of the analytical process is essential to achieve accurate cholesterol values.* Currently, the cholesterol values of commercial reference materials are generally assigned by instrument-method systems in nonstandardized clinical laboratories without traceability to the reference method at CDC. This lack of uniformity by the manufacturers in the development of dependable calibration materials with accurate assignment values is one of the major factors contributing to inaccuracy in cholesterol measurements.

It is essential that cholesterol values, especially for calibration materials and reference quality control materials, be assigned according to a procedure that traces back to the National Reference System for Cholesterol (see page 19).

Other major factors that contribute to *accuracy* are reagent systems that are based on good analytical chemistry principles, which means that the assay method can produce a ***true value***, if it:

- is specific for the measurement of cholesterol, and not other sterols;
- measures cholesterol completely;
- is not influenced by interfering biological substances, such as vitamin C, bilirubin, hemoglobin, or lipemia;
- is insensitive to matrix effects, i.e., the influence of lyophilized materials or spiked samples; and
- is linear within a specified range of concentration.

These factors and matrix specifications for the development of reliable calibration and quality control materials will be discussed in greater detail in the subsequent report of the Laboratory Standardization Panel.

G. *Resources Currently Available*

CDC distributes, on a limited basis, through the CDC-NHLBI Lipid Standardization Program, frozen serum pools prepared from human-based materials. These time-tested, reliable reference materials are available to manufacturers and specialized lipid research laboratories, but not to the general laboratory community. Target values are assigned by the reference method for cholesterol, which is based upon the modification of the Abell et al. method (20,21). The accuracy of the *CDC reference method* is traceable to the accepted *NBS definitive method*, an isotope-dilution-mass-spectrometry procedure (26), and *certified reference material* (NBS/SRM 911). These two methods agree within 1.5% of each other (27). NBS offers for a nominal charge a *certified reference material* (NBS/SRM 909a)[2] with a definitive cholesterol method value of 143 mg/dL (3.70 mmol/L). This material is available for all clinical laboratories. The SRM/909a, a human-based lyophilized serum reference material, may be utilized to assess whether a particular assay system is accurate or not. NBS will soon offer certified reference materials of frozen specimens at three cholesterol levels with *reference* and *definitive* method target values. Currently, the CAP[3] also has available tri-level human-based lyophilized reference materials with target values assigned by the *NBS definitive method*.

These efforts to improve performance, achieve uniformity, and standardize results of the measurement of cholesterol are by no means unique. For over a decade now, there has been growing involvement of the clinical laboratory community in developing standardized guidelines leading to improvements and better accuracy in the measurements of many blood constituents. At a conference held in 1977, the concept of a national reference system to provide an *accuracy* base for analytes such as cholesterol was enthusiastically supported by representatives of the clinical laboratory community (28). This conference, "A National Understanding for the Development of Reference Materials and Methods for Clinical Chemistry," was sponsored by CDC, the Food and Drug Administration, and NBS and co-sponsored by professional societies and industrial trade associations. The conferees unequivocally endorsed the development of voluntary consensus standards for a hierarchy of high-accuracy interlocking reference materials and methods. As evidenced by a statement from the summary report, conferees supported these standards as a means to

> "assure that the result(s) from any clinical laboratory in the United States can be interchanged with those of any other clinical laboratory within the defined limits of *accuracy* and *precision* required to meet the needs of medical practice" (28).

2 Office of Standard Reference Materials, National Bureau of Standards, Gaithersburg, MD 20899.
3 College of American Pathologists, 5202 Old Orchard Road, Skokie, IL 60077-1034.

The National Committee for Clinical Laboratory Standards (NCCLS) was charged to create and manage a National Reference System for the Clinical Laboratory (NRSCL) through a council whose members represent the major professional, governmental, and industrial organizations of the United States. The NRSCL Council has approved the NBS *definitive method* and the CDC *reference method* as the accuracy base for serum cholesterol measurements in the United States, which together with the NBS *certified reference material* (NBS/SRM 911) and the CDC *reference materials*, are recognized as the *National Reference System for Cholesterol* (document R53-P).[4]

The adoption of uniform cholesterol cutpoints now mandates more stringent requirements for precision and accuracy of measurement. The utilization of the NCCLS National Reference System for Cholesterol will assist the organized national effort to assure and monitor reliable cholesterol determination in the laboratories. In order to realize these goals, the Laboratory Standardization Panel recommends the standardization of cholesterol measurement in all U.S. clinical laboratories.

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4 National Committee for Clinical Laboratory Standards, 771 East Lancaster Avenue, Villanova, PA 19085.

Glossary

Accuracy: The degree of agreement of a measurement (or an average of measurements of the same thing), X, with an accepted reference or true value, T, usually expressed as the difference between two values, X-T, or the difference as a percentage of the reference or true value, 100 (X-T)/T.

Analyte: The constituent of the specimen to be measured; the element, ion, compound, substance, factor, infectious agent; or enzymatic, hormonal, or immunological activity; or any thing or property whose presence or absence, concentration, or activity is to be determined.

Bias: A quantitative measure of inaccuracy or departure from accuracy. A signed difference between two values. In general, the difference between the true, accepted, or expected value and the observed value; expressed in the units of the measurement or as a percentage.

Calibrator: A material, solution, or lyophilized preparation designed to be used in calibration. The values or concentrations of the analytes of interest in the material are known within limits ascertained during its preparation, and confirmed in use.

CDC Confirmatory Value: The mean concentration obtained from 12 in-control analyses by CDC using the Abell-Kendall based cholesterol reference method. This mean has a 95% chance of being within 1% of the true Abell-Kendall value for the method.

Certified Reference Material (CRM): A reference material that is accompanied by or is traceable to a certificate or publication issued by an organization that is generally accepted as competent and which states the values of the properties concerned.

Coefficient of Variation: A measure of precision calculated as the standard deviation of a set of values divided by the average. It's usually multiplied by 100 to be expressed as a percentage.

Definitive Method: An analytical method that has been subjected to in-depth investigation and evaluation for sources of inaccuracy and imprecision, including nonspecificity. The best estimates of bias and standard deviations throughout the analytical range, as well as the analytical specificity of the process, are all of a magnitude compatible with the intended use of the process, and all of these are stated explicitly in the method along with their uncertainty interval. Unless directed otherwise in the method, the mid-point of the uncertainty intervals can be taken as the true value.

Matrix: The environment surrounding a given analyte; for example serum.

Outlier: A result which is so far from the expected value that it does not appear to be part of the same population. Statistical tests are used to identify outliers.

Peer Group: A group of at least 20 laboratory participants in the CAP Survey using the same method-instrument combination.

Precision: Freedom from inconsistency or random error. For repeated measurement of any given specimen the random errors are generally assumed to be distributed normally about the observed mean.

Primary Standard: A reference material that is of fixed and known chemical composition and capable of being prepared in essentially pure form. Alternatively: Any certified reference material that is generally accepted or officially recognized as the unique standard for the assay regardless of its level of purity of analyte content.

Proficiency Testing: A program in which specimens of quality control materials are periodically sent to members of a group of laboratories for analysis and comparison of each laboratory's results with those of other laboratories in the group through some central organization.

Quality Control Material: A material, solution, lyophilized preparation, or pool of collected serum designed to be used in the process of maintaining control of performance. The concentrations of the analyte of interest in the control material are known within limits ascertained during the preparation, and confirmed in use.

Reference Material: A material or substance one or more properties of which are sufficiently well established for use in calibrating a process or for use in quality control. Its characterization may be more stringent than most working calibrators or controls.

Reference Method: An analytical method whose accuracy and precision are sufficient as demonstrated by direct comparison with the definitive method and whose low incidence of susceptibility to known interferences is thoroughly documented so that the stated purpose of the analytical process can be achieved.

Reference Value: A statement of the accepted value for some analyte in some matrix which has been determined by analysis employing a reference method.

Reliability of Measurements: This involves two components: Precision is the within-day or day-to-day reproducibility; Accuracy is the true value that is traceable to a reference or definitive method.

Secondary Standard: A reference material, the analyte concentration of which has been ascertained by reference to a primary standard.

Standard Reference Material (SRM): A material produced in quantity, of which certain properties have been certified by the National Bureau of Standards to the extent possible to satisfy its intended use.

Standardization: A collaborative and interactive process to meet a specific level of analytical performance, and to reduce among-laboratory variation to produce comparable laboratory results.

Systematic Bias: A consistent difference between the value obtained and that accepted as true or expected. Estimated independently of random error by averaging replicates. Expressed in the units of the method calculated as the average difference between the values expected and obtained.

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